

OVARIAN DEVELOPMENT AND SEX PHEROMONE TITER IN THE SMALLER TEA TORTRIX MOTH *ADOXOPHYES* SP. (LEPIDOPTERA: TORTRICIDAE)¹

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Rong Kou, Yien-Shing Chow and Chien-Chung Cheng (1992) Ovarian development and sex pheromone titer in the smaller tea tortrix moth *Adoxophyes* sp. (Lepidoptera: Tortricidae). *Bull. Inst. Zool., Academia Sinica* 31(2): 105-110. The temporal relationship between ovarian development and pheromone titer was investigated in the smaller tea tortrix moth, *Adoxophyes* sp. (Lepidoptera: Tortricidae). At each chronological age, females that called for the first time were sacrificed at the end of the scotophase for pheromone titer analysis, oocyte measurement, and egg count. The ovaries of females that initiated calling on days 2-3 were at a similar developmental stage, but were significantly more mature than those of females calling for the first time on day 1. On the other hand, pheromone titer in first-time callers decreased with increasing chronological age.

Key words: Ovarian development, Pheromone titer, *Adoxophyes* sp. Lepidoptera, Tortricidae.

In Lepidoptera, the association between oocyte maturation and calling behavior has been studied in the light brown apple moth, *Epiphyas postvittans* Walker (Lawrence and the Bartell, 1972), the black cutworm, *Agrotis ipsilon* Hufnagel (Swier *et al.*, 1976) and the bertha armyworm, *Mamestra configurata* Walker (Howlader and Gerber, 1986; Gerber and Howlader, 1987). Recently, a strong association between oocyte maturation and pheromone titer was reported in migratory armyworm moths, *Pseudaletia unipuncta* Haworth (Cusson and McNeil, 1989b); juvenile hormone (JH) was also shown to be essential to the initiation of both calling behavior and pheromone pro-

duction (Cusson and McNeil, 1989a).

In our previous study, the sex pheromone of the smaller tea tortrix moth, *Adoxophyes* sp. (Lepidoptera: Tortricidae) in Taiwan was identified as Z11-14:OAc/Z9-14:OAc=64/36 (Kou *et al.*, 1990). For the present study, we investigated the temporal relationship between oocyte development and pheromone titer in *Adoxophyes* sp. as a basis for the further study of the physiological regulation of pheromone production and calling behavior.

MATERIALS AND METHODS

Insects

Female smaller tea tortrix moth pupae

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were obtained from the Taiwan Tea Experiment Station, Pwu-Sin, Taiwan, and maintained at 24–26°C under 14:10 (L:D) photoperiod. Emerged moths were fed a 10% aqueous sucrose solution. Most adults emerged just after the onset of a photophase and only these moths were used for the test.

Calling behavior, ovarian development, and pheromone titer analysis in first-time callers

Starting from the first night following emergence, a total of 171 females were continuously observed for the last 2 h of the scotophase (the period when calling is initiated by females for the first time) for 4 consecutive days under a 5-W red lamp; the number of females that called for the first time on that date was recorded to determine the percentage of first-time callers at each chronological age. Calling females are easily recognized by their extruded ovipositors and vibrated wings (Kou *et al.*, 1990). At the end of each scotophase, the pheromone gland of each female calling for the first time on that date was excised for pheromone titer analysis; the females were then placed in individual containers for later assessment of ovarian development. Females were observed for four days after emergence during which 88% initiated calling; none called at 5 days after emergence.

Titers of (*Z*)-11-tetradecenyl acetate (*Z*11-14:OAc) and (*Z*)-9-tetradecenyl acetate (*Z*9-14:OAc)—the sex pheromone components of *Adoxophyes* sp. females (Kou *et al.*, 1990)—were determined by soaking the moths' last two abdominal segments in 5 μ l hexane containing 20.0 ng (*Z*)-11-hexadecenyl acetate (internal standard) for 20 min. Analyses were conducted on a Carbowax 20 M \times 0.25 mm ID fused silica capillary column, using a Shimadzu 14-A gas chromatograph equipped with a flame ionization detector.

Chromatographic conditions: nitrogen carrier 0.5 kg/cm², and column temperature isothermal at 190°C.

To assess ovarian development, each female ovary was dissected and stained for 30 min in Grenacher borax carmine (Humason, 1972). The ovary was then washed twice in 70% ethyl alcohol, after which—if eggs were present—red-stained oocytes were discarded. Unstained eggs were counted, and basal oocyte widths were measured using an ocular micrometer.

Data analysis

The Kruskal-Wallis Analysis for Multiple Comparison was used to detect mean differences within calling categories (Siegel and Castellan, 1989). In addition, a Spearman Rank-order Correlation Analysis (Siegel and Castellan, 1989) was performed to determine relationships between ovarian development and pheromone titer.

RESULTS

Calling behavior

The average age at which *Adoxophyes* sp. initiated calling at 24–26°C was 1.81 ± 0.75 days following emergence; however, there was considerable variability (Fig. 1). Most females (74%) initiated calling within two days after emergence, but rarely initiated calling after the third day.

Ovarian development in first-time callers

In first-time callers, ovarian maturation progressed with age (Fig. 2). When basal oocyte widths were compared, 1-day-old first-time callers had significantly smaller basal oocytes (0.32 mm) than 3- or 4-day-old first-time callers (0.37 mm and 0.38 mm, respectively) ($p < 0.05$, Kruskal-Wallis Analysis). Similarly, when the

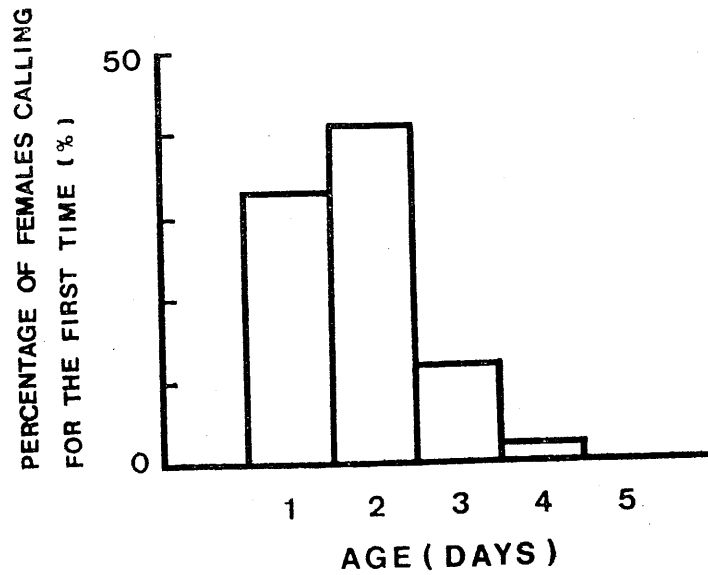


Fig. 1. Age following emergence at which female *Adoxophyes* sp. call for the first time ($n=171$).

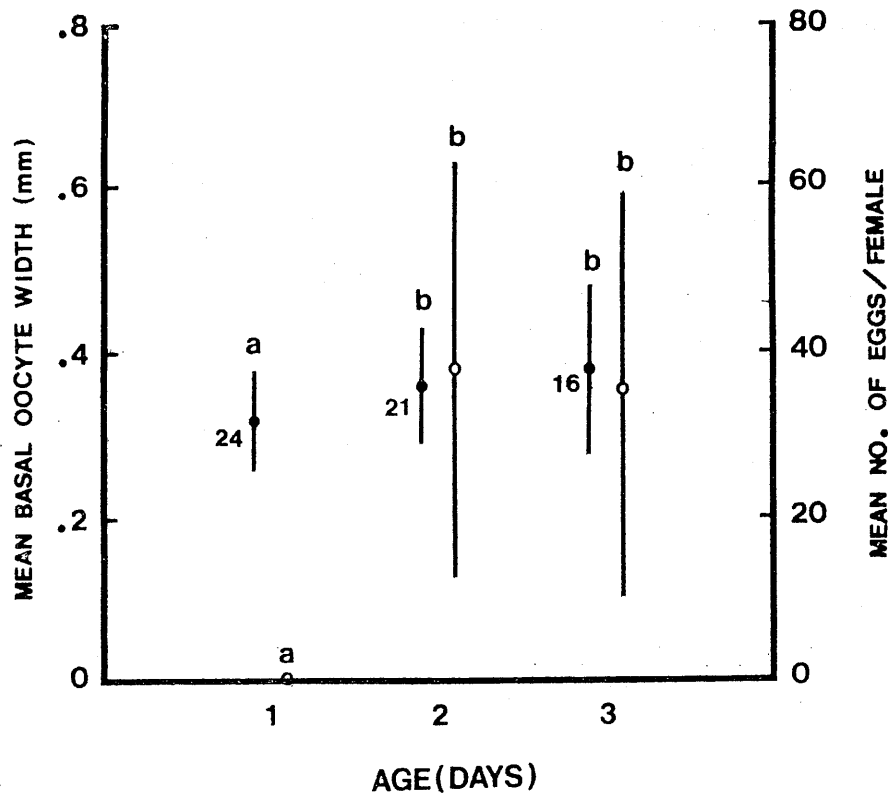


Fig. 2. Mean (\pm SE) basal oocyte width and number of eggs in 1- to 3-day-old first-time callers. Numbers beside means are sample sizes. Means followed by the same letters are not significantly different at the $p=0.05$ level (Kruskal-Wallis Analysis).

●: basal oocyte width; ○: number of eggs.

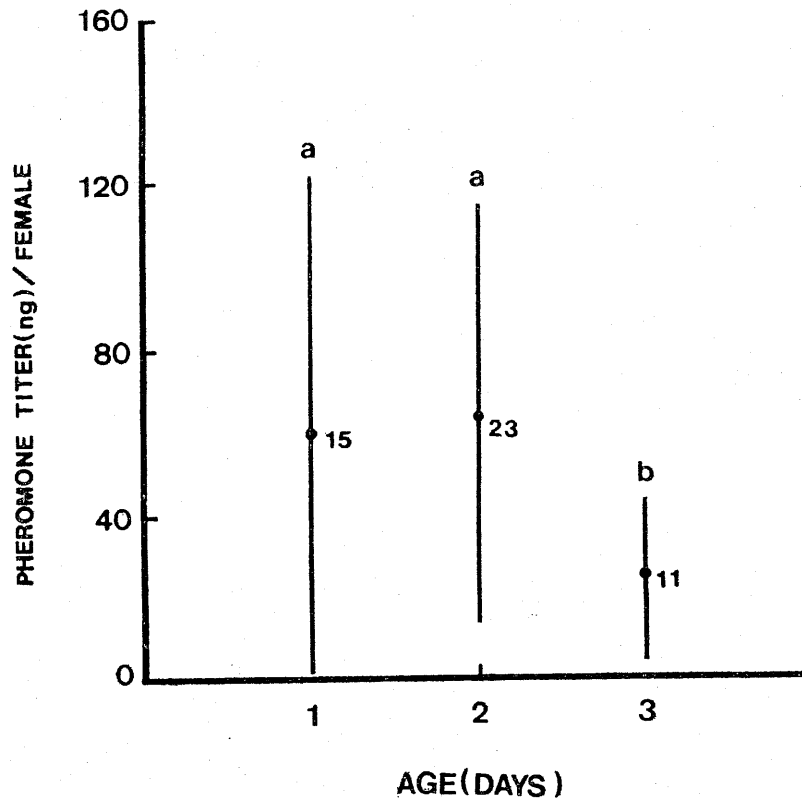


Fig. 3. Mean (\pm SE) pheromone titer in 1- to 3-day-old first-time callers. Number beside means are sample sizes. Means followed by the same letters are not significantly different at the $p=0.05$ level (Kruskal-Wallis Analysis).

number of mature eggs in ovaries were compared, 1-day-old first-time callers had no mature eggs, but 2- and 3-day-old first time callers had significantly more mature eggs ($p < 0.05$, Kruskal-Wallis Analysis). No significant difference was found between 2- and 3-day-old first-time callers.

Pheromone titer in first-time callers

Pheromone titer analysis of first time callers revealed trends reverse to those found with ovarian development (Fig. 3); 1- and 2-day-old first-time callers had significantly higher pheromone titers (61.1 ng/female and 64.5 ng/female, respectively) than 3-day-old first time callers (22.6 ng/female) ($p < 0.05$, Kruskal-Wallis Analysis).

When callers were ranked by basal oocyte width or number of eggs, no correlation was found between basal oocyte width and pheromone titer, nor between the number of eggs and pheromone titer. However, a positive correlation was found between basal oocyte width and number of mature eggs ($r = 0.23$, $p < 0.005$, Spearman Rank-order Correlation Analysis).

DISCUSSION

Individual variability in initiation of female calling following emergence was reported for many Lepidopteran moths (Kou and Chow, 1987; Lawrence and Bartell, 1972; Turgeon and McNeil, 1982; West *et al.*, 1984). This variability may

be related to differences in reproductive maturation rates (Fitt, 1989; Swier *et al.*, 1976).

In *Adoxophyes* sp., ovarian development in first-time callers increased significantly with increasing chronological age; but on the other hand, pheromone titer decreased with increasing chronological age; younger first-time callers had higher pheromone titers which may increase their chances of mating by being the first to attract males. The pheromone communication system of these female moths seems to develop more quickly than the ovary. The ecological and physiological implication of this phenomenon need further study.

In Lepidoptera, a positive relationship between ovarian maturation and the pheromonal communication system was investigated in *Pseudaletia unipuncta* (Cusson and McNeil, 1989b); juvenile hormone (JH) was shown to be essential to the initiation of both calling behavior and pheromone production. It has been proposed that JH acts directly on the central nervous system to allow the release of PBAN (pheromone biosynthesis activating neuropeptide) (Jaffe *et al.*, 1986; Raina *et al.*, 1987); this release would trigger pheromone biosynthesis and the sending of neural messages initiating calling behavior (Cusson and McNeil, 1989a).

Juvenile hormone regulates oocyte growth and migratory behavior in many insect species (Karlinsky, 1967; Nijhout and Riddiford, 1974; Barker and Herman, 1976; Rankin *et al.*, 1986); however, in the present study no obvious relationship was found between ovarian maturation and the pheromone communication system in *Adoxophyes* sp.. The results of our study—that most females called within the first two days following emergence, and that pheromone titers decreased with increasing chronological age—may be

results of their short adult lives (about 6–8 days); in addition, our results may indicate that *Adoxophyes* sp. is not a migratory species, therefore the pheromone production system need not be rested for a period of time prior to migratory behavior. Consequently, JH may not have much effect on pheromone production. Mechanisms possibly involved in both the calling behavior and pheromone production system of this species will be further investigated.

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茶姬捲葉蛾 (*Adoxophyes* sp.) 卵巢發育及其性費洛蒙含量

寇 融 周 延 鑫 程 建 中

本研究探討茶姬捲葉蛾卵巢發育及其性費洛蒙含量之間的關係。不同日齡的求偶雌蛾均做單隻的性費洛蒙定量測定，並檢視其卵寬及成熟卵粒數。2~3日齡的求偶雌蛾卵巢發育程度相似，但顯著較1日齡求偶雌蛾為成熟。另一方面，求偶雌蛾之性費洛蒙含量隨著日齡增加而下降。